HPLC METHODS FOR PHARMACEUTICAL ANALYSIS

Volumes 2-4

George Lunn

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ACKNOWLEDGMENTS

These days the production of a technical book is a complex process requiring the talents of many people. Even by modern standards, however, this was a particularly involved process. The raw data initially consisted of thousands of individual files, one for each abstract. These files were converted into a database that was used to make the CD for the electronic version as well as prepare the coded report that was used to set the galley and page proofs. The people who worked on this project are as follows:

For Synexchem Consulting Services International LLC: Software Developers Peter Baričič and Martin Mackov; Abstracters Elena Belajová, Research Institute of Foods; Alica Čániová, Slovak Technical University, Department of Analytical Chemistry; Katarina Hroboňová, Slovak Technical University, Department of Analytical Chemistry; Gabriela Kristofiková; Pavol Kubalec, Slovak Technical University, Department of Analytical Chemistry; Gabriela Machalová; Martina Nahálková; Mária Tegzová, Drug Research Institute; and Pavol Valachovič, Drug Research Institute

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The use of the National Library of Medicine, National Institutes of Health Library, and the FDA Medical Library is greatly appreciated. Although many people have helped with the preparation of this work, the mistakes are my own. I would appreciate hearing from anyone who has corrections, comments, or suggestions. I can be reached at lunng@cder.fda.gov.

The content of this volume does not necessarily reflect the views or policies of the Food and Drug Administration, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

ABOUT THIS BOOK

SCOPE

A computer search was used to identify relevant references. The search was conducted using Medline, 1980 to date. Since references from the *Journal of Liquid Chromatography* are not included in Medline, these references were manually added to the database.

The search strategy was as follows: HPLC (tw) **or** HPLC (mh) **or** liquid chromatography (mh) **and** USAN drug name (tw or mh), where tw = text word and mh = MESH heading.

In addition to the Medline search, some journals were routinely surveyed for relevant articles. These journals were:

American Journal of Health-System Pharmacy
Analyst
Analytical Chemistry
Antimicrobial Agents and Chemotherapy
Arzneimittelforschung
Biological and Pharmaceutical Bulletin
Biomedical Chromatography
Biopharmaceutics and Drug Disposition
Chemical and Pharmaceutical Bulletin
Chromatographia
Clinical Chemistry
Clinical Pharmacology and Therapeutics
Drug Metabolism and Disposition
Farmaco
Journal of Analytical Toxicology
Journal of AOAC International
Journal of Chromatographic Science
Journal of Chromatography, Part A and Part B
Journal of Clinical Pharmacology
Journal of Forensic Sciences

Journal of Liquid Chromatography and Related Technology
Journal of Medicinal Chemistry
Journal of Pharmaceutical and Biomedical Analysis
Journal of Pharmaceutical Sciences
Journal of Pharmacology and Experimental Therapeutics
Pharmaceutical Research
Pharmazie
Therapeutic Drug Monitoring
Xenobiotica

Many other journals were consulted when relevant articles were identified by computer searches.

CHAPTER STRUCTURE

Each chapter is headed by the name and structure of the target compound as well as other useful data such as the CAS Registry Number, molecular formula, and molecular weight. In general the United States Adopted Name (USAN) is used throughout to identify each drug, although we sometimes use a truncated version of this name, e.g., naproxen for naproxen sodium. Exceptions are clavulanate potassium, which is listed in the "Clavulanic Acid" monograph, EDTA, which is used in preference to edetic acid, hyoscyamine, which is listed in the monograph for its racemate atropine, isotretinoin and tretinoin, which are listed in the monograph "Retinoic Acid," levonorgestrel, which is listed in the "Norgestrel" monograph, and some of the steroids, which are listed for convenience in the monograph "Estrogens, conjugated." Names of derivatives, such as esters, which would have different chromatographic properties, are identified by placing the derivative name in parentheses after the retention time.

Reference is also made at the head of each chapter to the relevant abstract in the *Merck Index*. **Note that these numbers refer to the 12th edition of the Merck Index.**[1] Much useful information, such as melting point, solubility, optical rotation, and references to reviews, can be found in the *Merck Index*. In addition, the relevant sections in the series *Organic Chemistry of Drug Synthesis*, by Lednicer and Mitscher,[2–6] are referenced. These books give valuable information about the syntheses of various drugs and this may be helpful in determining impurities, understanding degradation reactions, and so on.

ABSTRACT STRUCTURE

The detailed procedures given normally contain the following sections. Of course, not all papers give full details, so some sections may be missing.

Matrix
Sample Preparation
Guard Column
Column
Mobile Phase
Flow Rate

Injection Volume	Also
Retention Time	Noninterfering
Detector	Interfering
Internal Standard	Limit of Detection
Column Temperature	Limit of Quantitation
Extracted	Key words
Simultaneous	Reference

ABSTRACT CONVENTIONS

Also	Compounds that can be analyzed at the same time. It is not specified whether they interfere, but they can be extracted. See also Extracted, Simultaneous.
Column	Dimensions are length (mm) × internal diameter (mm), and the material is stainless steel unless otherwise indicated.
Column Temperature	If other than ambient (all temperatures are in degrees C)
Derivatization	Pre-column unless otherwise mentioned (in Key Words)
Detector	Wavelengths in nm
Extracted	Compounds that can be extracted from the matrix in question and analyzed at the same time and do not interfere. See also Also, Simultaneous.
Flow Rates	In mL/min.
Guard Column	Dimensions are length (mm) × internal diameter (mm)
Impurities	If method resolves compound and impurities (in Key Words)
Injection Volume	In microliters (μL). Injection Volume may be either the volume actually injected or the volume of the injection loop. If it is the volume actually injected, this value is also given in the Sample Preparation section. If the actual injection volume is not given in the Sample Preparation section, the Injection Volume given is that of the injection loop.
Interfering	Compounds that interfere with the analysis of the target compound. Compounds which interfere with the chromatography of the internal standard (IS) are listed under simultaneous (another IS can always be selected or an external standard procedure can be used).
Matrix	A controlled vocabulary is used (see below)
Metabolites	If method resolves compound and metabolites (in Key Words)
Mobile Phase	Ratios are v/v and gradients are linear unless otherwise noted. Times given when describing gradient elution, and other procedures such as column switching, are the times for each step, e.g., "MeOH:water 15:85 for 4 min, to 50:50 over 2 min, maintain at 50:50 for 4 min." If we were to include the cumulative times (t) in the example above, it would read: "MeOH:water 15:85 for 4 min (t = 4), to 50:50 over 2 min (t = 6), maintain at 50:50 for 4 min (t = 10)."
Noninterfering	Compounds which do not interfere with the analysis for various reasons, e.g., they are not extracted, they are not detected.

Retention Time	This is frequently estimated from a reproduced chromatogram and so the accuracy may not be great (in minutes).
Simultaneous	Compounds which can be analyzed at the same time and do not interfere. Note that the compound cannot necessarily be extracted from the matrix in question (although it may be). See also Also, Extracted.
SPE	For the sake of consistency, conditioning procedures for solid-phase extraction (SPE) cartridges are always described at the beginning of the sample preparation sections. Bear in mind, however, that the conditioning procedure should be carried out just prior to use. Thus, if sample preparation is a lengthy procedure, it may be necessary to delay SPE cartridge conditioning until the step requiring the cartridge.
Species	If other than human; noun is used instead of adjective, e.g., cow not bovine. In some cases, human may be specified. For example, if **both** human blood and rat blood are analyzed, **both** human and rat will be indicated (in Key Words).

MATRIX

To help with searching, a controlled vocabulary is used to limit the number of terms in the matrix section. For example, the term raw materials is not used, the term bulk is used instead. In a number of cases the matrix is associated with various key words which can be used to narrow the search. For example, the matrix term blood has the key words plasma, serum, and whole blood associated with it. Thus, if you are interested in the determination of the drug in blood in general you should search the matrix field for blood. If, however, you are specifically interested in finding the drug in plasma you should search the key words field for plasma.

Matrix	Associated Key Words
bile	
blood	plasma, serum, whole blood
bulk	
CSF	
formulations	capsules, creams, injections, ointment, tablets, etc.
microsomal incubations	
milk	
perfusate	
reaction mixtures	
saliva	
solutions	buffer, water
tissue	brain, heart, kidney, liver, muscle, spleen, etc.
urine	

ABBREVIATIONS

BHT	2,6-di-tert-butyl-4-methylphenol, butylated hydroxytoluene

CE	capillary electrophoresis
DMSO	dimethyl sulfoxide
E	electrochemical detection
em	emission wavelength
EtOH	ethanol
ex	excitation wavelength
F	fluorescence detection
FW	formula weight
GPC	gel permeation chromatography
h	hour
HPLC	high-performance liquid chromatography
IS	internal standard
L	liter
LOD	limit of detection or some other description indicating that this is the smallest concentration or quantity that can be detected or analyzed for
LOQ	lower limit of quantitation, either given as such in the paper or taken as the lower limit of the linear quantitation range
M	molar (i.e., moles/L)
MeCN	acetonitrile
MeOH	methanol
min	minutes
mL	milliliter
mM	milli-molar (i.e., milli-moles/L)
MTBE	methyl tert-butyl ether
nM	nano-molar (i.e., nano-moles/L)
psi	pounds/sq. in. (1 psi = 6.89476 kPa)
s	seconds
SEC	size exclusion chromatography
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIM	selected-ion monitoring
SPE	solid phase extraction
Temp	temperature
U	units
UV	ultraviolet detection

WORKING PRACTICES

In general, good working practice, e.g., using high-grade materials, is assumed. Solutions containing compounds should be protected from light and silanized glassware should be used unless you have good reason to believe that these precautions are not necessary. Details of solution preparation are generally not given. It should be remembered that the preparation of a dilute aqueous solution of a relatively water-insoluble compound can frequently be made by dissolving the compound in a small volume of a water-miscible organic solvent and diluting this solution with water. A number of excellent texts[7–9] discuss good working practices and procedures in HPLC and these should be consulted.

It is also assumed that safe working practices are observed. Organic solvents should only be evaporated in a properly functioning chemical fume hood, correct protective equipment should be worn when dealing with potentially hazardous biological materials, and waste solutions should be disposed of in accordance with all applicable regulations.

A number of solvents are particularly hazardous. For example, benzene is a human carcinogen[10]; chloroform,[11] dichloromethane,[12] dioxane,[13] and carbon tetrachloride[14] are carcinogenic in experimental animals; and DMF[15] and MTBE[16,17] may be carcinogenic. Organic solvents are, in general, flammable and toxic by inhalation, ingestion, and skin absorption. Sodium azide is carcinogenic and toxic and liberates explosive, volatile, toxic hydrazoic acid with acid. Sodium azide can form explosive heavy metal azides, e.g., with plumbing fixtures, and so should not be discharged down the drain.[18] Disposal procedures have been described for a number of hazardous drugs and reagents[18] and recent papers describe a procedure for the hydrolysis of acetonitrile in waste solvent to the much less toxic acetic acid and ammonia.[19,20] Recent work has shown that n-hexane is surprisingly toxic.[21]

PIC REAGENTS

These reagents are offered by Waters as buffered solutions containing the following compounds:

PIC A is tetrabutylammonium sulfate
PIC B5 is pentanesulfonic acid
PIC B6 is hexanesulfonic acid
PIC B7 is heptanesulfonic acid
PIC B8 is 1-octanesulfonic acid
PIC D4 is dibutylamine phosphate

SUPPLIERS

Suppliers of critical items such as columns are given in the abstracts but the suppliers for widely available items are not listed. These suppliers are as follows:

Item	Supplier
Adsorbosphere	Alltech Associates
Asahipak	Asahi Chemical
Bakerbond	J.T. Baker
Bond Elut	Varian
μBondapak	Waters
Chiralcel	Daicel
Co:Pell	Whatman
Corasil	Waters
Cyclobond	Advanced Separation Technologies
Econosil	Alltech Associates
Econosphere	Alltech Associates

Extrelut	E. Merck
Hypersil	Shandon
Inertsil	MetaChem
LiChroprep	E. Merck
LiChrosorb	E. Merck
LiChrosphere	E. Merck
Micropak	Varian
Microsorb	Rainin
NewGuard	Applied Biosystems
Nova-Pak	Waters
Nucleosil	Macherey Nagel
Partisil	Whatman
Pecosphere	Perkin-Elmer
Porasil	Waters
Sep-Pak	Waters
Spheri-5	Applied Biosystems
Spheri-10	Applied Biosystems
Spherisorb	Phase Separations
SPICE	Analtech
Supelcosil	Supelco
Ultrasphere	Beckman
Ultremex	Phenomenex
Vydac	The Separations Group
Zorbax	Mac-Mod Analytical

This list is not intended to be definitive. Many other companies supply these pieces of equipment.

TRADEMARKS

The following trademarks are used:

Trademark	Company
Adsorbosphere	Alltech Associates, Inc.
Asahipak	Asahi Chemical Industry Co. Ltd.
Bakerbond	J.T. Baker
Bond Elut	Varian Associates, Inc.
μBondapak	Waters Associates, Inc.
Chiralcel	Daicel Chemical Industries, Ltd.
Co:Pell	Whatman Chemical Separation Co.
Corasil	Waters Associates, Inc.
Cyclobond	Advanced Separation Technologies, Inc.
Econosil	Alltech Associates, Inc.
Econosphere	Alltech Associates, Inc.
Extrelut	E. Merck
Hypersil	Shandon Scientific, Ltd.
Inertsil	GL Sciences Inc.
LiChroprep	E. Merck

Trademark	Company
LiChrosorb	E. Merck
LiChrosphere	E. Merck
Micropak	Varian Associates, Inc.
Microsorb	Rainin Instrument Co. Inc.
NewGuard	Applied Biosystems
Nova-Pak	Waters Associates, Inc.
Nucleosil	Macherey Nagel
Partisil	Whatman Chemical Separation Co.
Pecosphere	Perkin-Elmer
PIC	Waters Associates, Inc.
Porasil	Waters Associates, Inc.
Resolve	Waters Associates, Inc.
Sep-Pak	Waters Associates, Inc.
Spheri-5	Applied Biosystems
Spheri-10	Applied Biosystems
Spherisorb	Phase Separations, Ltd.
SPICE	Analtech
Supelcosil	Supelco, Inc.
Ultrasphere	Beckman Instruments, Inc.
Ultremex	Phenomenex, Inc.
Vydac	The Separations Group
Zorbax	DuPont Company

REFERENCES

1. Budavari, S., Ed., *The Merck Index*, 12th ed., Merck & Co., Whitehouse Station, NJ, 1996.
2. Lednicer, D. and Mitscher, L.A., *The Organic Chemistry of Drug Synthesis*, Wiley, New York, 1977.
3. Lednicer, D. and Mitscher, L.A., *The Organic Chemistry of Drug Synthesis*, Vol. 2, Wiley, New York, 1980.
4. Lednicer, D. and Mitscher, L.A., *The Organic Chemistry of Drug Synthesis*, Vol. 3, Wiley, New York, 1984.
5. Lednicer, D., Mitscher, L.A., and Georg, G.I., *The Organic Chemistry of Drug Synthesis*, Vol. 4, Wiley, New York, 1990.
6. Lednicer, D., *The Organic Chemistry of Drug Synthesis*, Vol. 5, Wiley, New York, 1995.
7. Snyder, L.R. and Kirkland, J.J., *Introduction to Modern Liquid Chromatography*, 2nd ed., Wiley, New York, 1979.
8. Lawrence, J.F., *Organic Trace Analysis by Liquid Chromatography*, Academic Press, New York, 1981.
9. Snyder, L.R., Kirkland, J.J., and Glajch, J.L., *Practical HPLC Method Development*, 2nd ed., Wiley, New York, 1997.
10. Lewis, R.J., Sr., *Sax's Dangerous Properties of Industrial Materials*, 8th ed., Van Nostrand-Reinhold, New York, 1992, pp. 356–358.
11. Reference 15, pp. 815–816.
12. Reference 15, pp. 2311–2312.

13. Reference 15, pp. 1449–1450.
14. Reference 15, pp. 701–702.
15. Reference 15, p. 1378.
16. Belpoggi, F., Soffritti, M., and Maltoni, C., Methyl-tertiary-butyl ether (MTBE)—a gasoline additive—causes testicular and lympho-haematopoietic cancers in rats. *Toxicol. Ind. Health* **11**, 119–149 (1995).
17. Mehlman, M.A., Dangerous and cancer-causing properties of products and chemicals in the oil refining and petrochemical industry: Part XV. Health hazards and health risks from oxygenated automobile fuels (MTBE): Lessons not heeded. *Int. J. Occup. Med. Toxicol.* **4**, 219–236 (1995).
18. Lunn, G. and Sansone, E.B., *Destruction of Hazardous Chemicals in the Laboratory*, 2nd ed., Wiley, New York, 1994.
19. Gilomen, K., Stauffer, H.P., and Meyer, V.R., Detoxification of acetonitrile–water wastes from liquid chromatography. *Chromatographia* **41**, 488–491 (1995).
20. Gilomen, K., Stauffer, H.P., and Meyer, V.R., Management and detoxification of acetonitrile wastes from liquid chromatography. *LC.GC* **14**, 56–58 (1996).
21. Meyer, V., A safer solvent. *Anal. Chem.* **69**, 18A (1997).